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In vitro and *in vivo* evaluation of gastroretentive floating drug delivery system of ofloxacin

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ABSTRACT

This study aimed to develop hydrophilic matrix based controlled release gastroretentive drug delivery system of ofloxacin and conducting its *in vitro* and *in vivo* evaluations. Effervescent floating gastroretentive drug delivery system of ofloxacin was prepared utilizing Box–Behnken statistical design with 3 factors, 3 levels and 15 experimental trials. Formulation optimization was done by setting targets on selected responses. *In vivo* studies were carried out for the optimized formulation with 12 healthy human volunteers and obtained pharmacokinetic parameters were compared with the marketed once daily formulation, "Zanocin OD". Optimized formulation showed satisfactory controlled *in vitro* drug release for more than 12 h with excellent buoyancy properties (floating lag time <1 min, floating duration >16 h). Optimized and marketed formulations were found to have similar *in vitro* release profile ($f2 = 79.22$) and also were found to be bioequivalent. Serum ofloxacin concentration was well maintained above its reported minimum inhibitory concentrations for most of the pathogens for sufficiently longer duration. C_{max} and AUC values of optimized formulation were found to be significantly higher than of marketed product despite their bioequivalence. Bettertherapeutic effect can be expected since ofloxacin exhibits concentration dependent killing. Hence, gastroretention can be a promising approach to enhance bioavailability of ofloxacin with narrow absorption window in upper GIT.

1. Introduction

The importance of controlled drug delivery systems that release drug over an extended period of time has long been recognized in the pharmaceutical field. Application of such controlled release technology to oral drug delivery system however has been limited because the actual time for effective drug delivery is restricted by gastrointestinal transit time. Gastric retention devices are designed to prolong the gastric residence time of oral controlled release dosage forms. They thus result in increased contact time for drugs that act locally, increased absorption of drugs that have absorption windows in

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upper part of gastrointestinal tract (GIT), and better absorption for drugs less soluble in the intestinal fluid [1]. Several approaches have been developed to achieve extended gastric residence time of the oral drug delivery systems such as bioadhesive system, swelling and expanding systems, floating systems and delayed gastric emptying devices. Amongst these methods, floating drug delivery system is preferred one that offers a simple and practical approach to achieve Gastroretention [2]. Floating dosage forms have a bulk density lower than that of gastric fluids and therefore remain buoyant on the stomach contents to prolong the gastric retention time [3–7].

Ofloxacin is widely used fluoroquinolone and has been reported as one among the five most frequently prescribed antibiotics in Nepal [8]. It is a broad spectrum antibiotic effective against wide range of Gram-negative and Gram-positive microorganisms. Biological half life of this drug is from 5 to 6 h due to which frequent administration is required. To avoid the drawbacks of frequent administrations such as plasma level fluctuations and patient non-compliance it is desirable to have a controlled release dosage forms of ofloxacin. It has been reported that bioavailability of ofloxacin is strongly dependent on the local physiology of GIT. It is readily soluble in the acidic environment of the stomach and thus is preferentially absorbed from the upper part of GIT [9]. In the alkaline environment of intestine, precipitation of the drug occurs decreasing its absorption.

This study was conducted with an aim to develop floating gastroretentive tablet formulation incorporating 400 mg ofloxacin into hydrophilic polymeric matrix which would release the drug in stomach and upper part of GIT in a controlled manner. Since ofloxacin has site-specific absorption from these regions, gastroretention of the dosage form will improve its oral bioavailability [10–12].

2. Materials and methods

2.1. Materials

Ofloxacin (Batch no. A5/206), hydroxypropyl methyl cellulose (HPMC) K100M (Batch no. HP121406 MC) and crosspovidone (Batch no. YPVPP09319040) were obtained from Nepal Pharmaceutical Laboratories Pvt. Ltd., Birgunj, Nepal as gift samples. Sodium bicarbonate ($NaHCO_3$), citric acid, polyvinyl pyrrolidone (PVP K-30), magnesium stearate, lactose and isopropyl alcohol were purchased from local suppliers. Marketed product, "Zanocin OD", (manufactured by Ranbaxy, India; Batch no. 2033597), used as a reference, was purchased from the local retail pharmacy.

2.2. Box–Behnken statistical design

One of the widely used response surface designs, a Box–Behnken statistical design with 3 factors, 3 levels, and 15 runs with triplicate center points was employed for the formulation of floating gastroretentive tablets of ofloxacin. Formulation design, optimization and other investigations were done using Statgraphics Centurion XV software from Stat-Point Technologies, USA, version 15.2.06. The independent variables or the factors were the amount of HPMC K100M (X_1), crosspovidone (X_2) and $NaHCO_3$ (X_3). Levels of these factors were set in the formulation design on the basis of the results of preliminary study and are coded as −1, 0, and +1 (Table 1). The responses selected were the cumulative percentage drug release at 2 h (Y_1), 8 h (Y_2) and 12 h (Y_3), floating lag time (Y_4) and the total floating time (Y_5). Formulation optimization was done by setting targets for these response variables.

2.3. Preparation of floating gastroretentive tablets

Tablets were prepared by conventional wet granulation method using HPMC K100M as a release retardant, crosspovidone as a swelling agents and $NaHCO_3$ as gas generating agent. Citric acid was also incorporated in the formulation to provide sufficiently acidic medium for $NaHCO_3$ to react and maintain buoyancy. The compositions of designed 15 formulations are listed in Table 2. All ingredients (except gas generating agents and magnesium stearate) were passed through sieve no. 60 and mixed in a polybag for 10 min and granulated using PVP K30 (in isopropyl alcohol). The wet mass was passed through sieve number 14 and dried in hot air oven at 50 °C for 1.5 h. Dried granules were mixed with remaining ingredients and compressed using 16-station rotary tablet press (Rimek Minipress-I, India) using 13 mm flat punch in order to obtain controlled release floating gastroretentive tablets containing 400 mg of ofloxacin.

2.4. In vitro *analysis*

2.4.1. In vitro *drug release study*

Drug release from the tablets was studied using USP dissolution apparatus (Electrolab TDT-081, India), type I (basket method). A tablet was placed inside a basket and immersed in a dissolution vessel ($n = 6$) containing 900 ml of 0.1 N HCl (pH 1.2), used as dissolution media at 37 ± 0.5 °C and stirred at a speed of 100 rpm. The amount of drug released after 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14 and 16 h was determined using UV–visible spectrophotometer (Shimadzu Corporation, 1601, Japan) at 294 nm. Release profiles of the designed formulations were compared with that of the marketed formulation, "Zanocin OD". Similarity and difference factors were calculated using appropriate formulas.

2.4.2. In vitro *buoyancy study*

The floating property of the tablets was visually determined in triplicate. The floating lag time and the total floating time were determined in the USP dissolution apparatus containing 0.1 N HCl (pH 1.2, maintained at 37 ± 0.5 °C). The time required

Table 1 – Independent variables and their levels in Box–Behnken design.

	Level (mg per tablet)		
	Low	Middle	High
X_1 (amount of HPMC K100M)	−1 (40)	0 (70)	+1 (100)
X_2 (amount of crosspovidone)	−1 (60)	0 (105)	+1 (150)
X_3 (amount of $NaHCO_3$)	−1 (60)	0 (75)	+1 (90)

Table 2 – The composition of 15 formulations as per Box–Behnken design.

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Ofloxacin	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400
HPMC K100M	40	70	100	70	100	100	70	100	70	40	40	70	70	40	70
Crosspovidone	60	105	105	150	105	150	105	60	150	105	105	60	105	150	60
$NaHCO_3$	75	75	60	60	90	75	75	75	90	60	90	90	75	75	60
Citric acid	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
PVP K-30	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Mag. Stearate	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Lactose	150	75	60	45	30	0	75	90	15	120	90	105	75	60	135
Total	810	810	810	810	810	810	810	810	810	810	810	810	810	810	810

for the tablet to rise to the surface of the medium and float was considered as floating lag time. The duration of time the dosage form remained constantly on the surface of medium was considered as the total floating time.

2.4.3. *Drug release kinetics*

In vitro release data were subjected to model fitting analysis to know the order of drug release by treating the data according to zero order, first order and Higuchi's release kinetic equations. Since these kinetic models are generally unable to explain the drug release mechanism from polymeric matrices that undergo swelling and/or erosion during dissolution process, the release data were further fitted into Ritger–Peppas empirical equation [7,13,14]. According to this equation,

$$\frac{M_t}{M_\infty} = Kt^n$$

Here, M_t/M_∞ is the fractional drug release at time t; K is the release rate constant and n is the diffusional exponent indicative of the release mechanism.

2.4.4. *Compatibility of ofloxacin with excipients*

To investigate the chemical interaction, Fourier Transformed Infra Red (FTIR) analysis of admixture of ofloxacin and the excipients used in the formulation were carried out over the range of 400–4000 cm^{-1} using FTIR spectrometer (Shimadzu, IR Prestige 21, se Japan). The spectra produced by the pure drug alone and in combination with excipients were compared to confirm the interaction.

2.5. In vivo *evaluation*

In order to ascertain the pharmacokinetic property and expected clinical efficacy, *in vivo* evaluation of the optimized formulation containing 400 mg ofloxacin was carried out. Open label, randomized, two treatments, two periods, two sequences, single dose, two ways crossover comparative bioavailability study was conducted using healthy human subjects. Prior ethical approval was taken from the Institutional Review Committee (IRC) of Kathmandu University School of Medical Sciences/Dhulikhel Hospital (IRC KUSMS), for carrying out this study (Protocol approval number: 34/11). Pharmacokinetic data of optimized formulation was compared with *in vivo* data of marketed extended release product of ofloxacin 400 mg (Zanocin OD, Batch no. 1760135).

Twelve healthy, non-smoking, adult Nepalese male volunteers were enrolled in the study. Written informed consent was obtained from each subject after adequate explanation of the objectives, methods and potential hazards of the study. All volunteers gave medical screening examination before dosing to establish their fitness to participate in the study. Enrolled volunteers were randomly divided into two groups. One group administered optimized formulation and another group administered marketed formulation, "Zanocin OD" on the first study day under fasting condition. After a washout period of 7 days, on the second study day, volunteers exchanged formulations. In both study days, 5 ml of pre-dose blood sample was collected 15 min before drug administration. Later blood sample was collected at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16 and 24 h of drug administration. Blood samples were collected in vacutainer tubes without anticoagulant. It was allowed to clot at room temperature for 20 min followed by centrifuging for 15 min at 5000 rpm. Serum was transferred into a separate serum container and was promptly frozen at −40 °C until assay.

2.6. *Drug analysis*

2.6.1. *Sample preparation*

To 180 μl of thawed human serum sample, 20 μl of ciprofloxacin (internal standard) was added and vortex mixed for 30 s. 600 μl of methanol was added to the tube. After mixing for another 1 min the tube was centrifuged at 5000 rpm for 5 min using a cooling centrifuge (Remi, India). 20 μl of filtered supernatant was injected into the HPLC column for analysis.

2.6.2. *Chromatographic conditions*

Serum samples were analyzed for ofloxacin with a validated bioanalytical method using HPLC (Shimadzu, Japan) comprising of a pump (LC-20AD), autosampler (SIL-20AD), photo-diode array detector (SPD-M20A) and a column oven (CTO-10 ASVP). The data collection and integration was accomplished using LC solutions software.

Separation was performed on a reverse phase CAPCELL PAK C18, MG type column (250 mm length, 4.6 mm internal diameter, 5 μm particle size) from Shiseido Fine Chemicals. Mobile phase consisting of acetonitrile and 0.0625% triethylamine in water (12.5: 87.5, pH adjusted to 2.5 with orthophosphoric acid) was delivered at a flow rate of 1.5 ml/min. The system was operated at wavelength of 294 nm and temperature of column oven was set at 40 °C.

The peaks of ofloxacin and ciprofloxacin were resolved with good symmetry. The retention time for ofloxacin and ciprofloxacin was 8.2 ± 0.8 and 9.3 ± 0.76 min respectively. The

typical assay run time was 12 min. The bioanalytical method was validated as per ICH guidelines.

The serum drug concentration–time data was subjected to non-compartmental analysis using a pharmacokinetic software, WinNonlin®, standard edition, version 5.2.1 (Pharsight Corporation, USA), to obtain various pharmacokinetic parameters. Relative bioavailability was determined as the ratio of $AUC_{0-\infty}$ of optimized formulation to the $AUC_{0-\infty}$ of marketed product.

3. Results and discussion

3.1. *Compatibility of ofloxacin with excipients*

FTIR study revealed absence of potential chemical interaction between the drug and excipients. The infrared spectrum of ofloxacin alone showed characteristic peaks at 3427, 3043, 2968, 2785, 1716, 1620, 1550, 1458, 1056 cm^{-1} [15]. All of these typical peaks of ofloxacin remained unaffected in the spectrum of ofloxacin in combination with HPMC K100M and also in presence of other excipients used in the formulation.

3.2. In vitro *buoyancy*

Most of the formulations were found to have good buoyancy properties (Table 3). They floated immediately upon immersion in to the media and remained floated for 16 h. $NaHCO_3$ and citric acid were employed as gas forming agents dispersed in the matrix and this formulation was found to be appropriate for achieving desired buoyancy characteristics. Addition of $NaHCO_3$ was found essential to ensure rapid floating. Furthermore, since the pH of the stomach is elevated under fed condition (~3.5), citric acid was also incorporated in the formulation to provide sufficiently acidic medium for $NaHCO_3$ to react. This will allow the system to float independent of the pH of the medium. Upon immersion, $NaHCO_3$ starts reaction immediately with the acidic dissolution media and added citric acid. This reaction generates sufficient amount of CO_2 which get entrapped and protected within the gel layer formed by hydration of HPMC K100M. This leads to decreased density of the tablet (reported as 1.004–1.010 g/cm^3), as a result of which the tablet becomes buoyant [2,3,5,11,16–18]. Buoyancy property is further facilitated by relatively good acid solubility of ofloxacin which causes faster penetration of dissolution media into the matrix. This in turn causes quicker initiation of reaction resulting in faster generation of CO_2 making the tablets more buoyant.

It has been reported in several literatures that the amount of $NaHCO_3$ is directly related to the floating lag time [3,11,19–21]. As the amount of $NaHCO_3$ increases, floating lag time decreases due to the generation of larger amount of effervescence. However in this study, quantity of HPMC K100M was found to have more prominent effect on floating characteristics instead. Formulation containing higher amount of HPMC K100M had better buoyancy property. Decreasing its amount decreased floating duration and increased floating lag time [20,22]. This effect is more noticeable in formulations containing lesser amount of $NaHCO_3$. When floating characteristics of the fifteen formulations were compared, a longer floating lag time and a shorter duration of floating were observed in formulations F1, F10, F11 and F14 containing least (40 mg per tablet) amount of HPMC K100M (Table 3). The matrix formed with lower concentration of this polymer seems not to be capable enough to hold the bubbles and float. Also there was quicker loss of tablet shape integrity in these formulations which have further worsened their buoyancy property.

3.3. In vitro *drug release*

In vitro drug release and buoyancy study revealed that values of responses for these 15 formulations varied markedly indicating strong relationship between responses and the factors (Table 3). The range of response Y_1 was from 28.84% in F12 to 61.64% in F10. Response Y_2 ranged from 53.98% in F12 to a

Table 3 – Observed responses in designed fifteen formulations.

Formulations	Responses[a]				
	Y_1 (%)	Y_2 (%)	Y_3 (%)	Y_4 (s)	Y_5 (h)
F1	50.62 ± 7.20	81.09 ± 7.81	96.00 ± 1.89	1.00 ± 00	1.00 ± 00
F2	34.89 ± 2.07	70.79 ± 5.49	85.61 ± 4.71	25.00 ± 5	16.00 ± 00
F 3	34.29 ± 7.38	70.12 ± 6.33	80.97 ± 2.04	1.00 ± 00	16.00 ± 00
F 4	39.93 ± 1.14	77.75 ± 3.97	92.34 ± 1.53	1.00 ± 00	16.00 ± 00
F 5	31.26 ± 4.03	65.11 ± 5.43	83.37 ± 5.79	1.00 ± 00	16.00 ± 00
F 6	29.91 ± 3.97	55.08 ± 4.46	68.99 ± 5.47	1.00 ± 00	16.00 ± 00
F 7	33.14 ± 3.64	69.95 ± 5.27	84.57 ± 4.87	1.00 ± 00	16.00 ± 00
F 8	39.51 ± 3.76	74.35 ± 6.62	79.57 ± 5.34	54.00 ± 7	16.00 ± 00
F 9	34.71 ± 2.48	69.11 ± 2.63	81.44 ± 3.27	1.00 ± 00	16.00 ± 00
F10	61.64 ± 3.51	96.70 ± 4.14	100.00 ± 00	95.00 ± 5	1.00 ± 0.15
F 11	48.89 ± 4.17	69.28 ± 4.96	82.97 ± 6.28	156.00 ± 10	0.50 ± 0.10
F 12	28.84 ± 4.13	53.98 ± 4.59	69.61 ± 4.95	1.00 ± 00	7.00 ± 0.27
F 13	37.16 ± 6.66	75.10 ± 4.38	90.63 ± 0.47	1.00 ± 00	16.00 ± 00
F 14	41.64 ± 2.77	75.15 ± 6.59	98.36 ± 1.88	22.00 ± 15	4.00 ± 0.23
F 15	31.28 ± 4.62	70.48 ± 1.92	88.31 ± 0.87	78.00 ± 12	4.00 ± 0.25

[a] Data is expressed as mean ± standard deviation.

maximum of 81.09% in F1. Similarly another response Y_3 was in the range of 68.99 in F6 to 100% in F10.

Majority of the formulations extended the drug release for 16 h. Drug release from the hydrophillic matrix tablet is known to be a complex interaction between diffusion, swelling, and erosion mechanisms. These processes are controlled by the hydration of HPMC, which forms the gel barrier through which the drug diffuses [23]. Higher polymer concentration increase the diffusion path length for the drug due to forming greater amount of gel which retard drug release from the formulation. The growth of erosion front, diffusion front, and swelling front decrease with the increase in polymer proportion because of the formation of a stronger gel layer, which make the entry of medium into the matrix difficult [24–27].

In the present formulation design, amount of HPMC K100M varied from 40 to 100 mg per tablet (5–12% of total tablet weight). Formulations F1, F10, F11 and F14, containing low concentration of HPMC K100M, gave high release rate of 50.62%, 61.64%, 48.89% and 41.64% respectively at the end of 2 h. Also these formulations completed drug release within a short time. When quantity of HPMC K100M was increased to 100 mg per tablet in formulations F3, F5, F6 and F8, release at the end of 2 h decreased down to 34.29%, 31.26%, 29.91% and 39.51% respectively. These formulations successfully extended drug release for sufficiently longer duration. At the end of 16 h drug release from formulations F3, F5, F6 and F8 were found to be 93.08, 93.02, 80.01 and 90.54% respectively.

It has been observed that high release occurred in all designed formulations at the end of 2 h. Marketed product also gave a high release rate of 37.08% at the end of 2 h. Such type of release could be due to the reason that the gel layer, which controls drug release needs sometime to become effective. Till the gel barrier is being formed, high rate of erosion occurs resulting in high initial drug release [23,27,28]. After some time release rate slows down as polymer hydration occurs.

In vitro release data were fitted in to different release kinetic models and it was observed that regression coefficient was highest for the Higuchi model (except for F10 & F11). The *n* values for all formulations, except F1, F10, F11 and F14, were found to be lying within the range of 0.45–0.89, indicating anomalous or non-Fickian type of diffusion to be the predetermining mechanism of drug release.

Mathematical relationships for the measured responses and the independent variables or factors were generated with the help of software Statgraphics Centurion XV and are shown in Equations (1)–(5). These equations represent the quantitative effect of variables (X_1, X_2, X_3) and their interactions on the response. Coefficient with more than one factor term and those with higher order terms represent interaction terms and quadratic relationship respectively. A positive sign represents synergistic effect, while a negative sign indicates antagonistic effect or an inverse relationship between the factors and response [29]. Correlation coefficient (r^2) for the equations indicates the percentage variability in model fitting for that particular variable. The adjusted r^2 value is more suitable for comparing models with different number of independent variables which can be obtained by including only statistically significant ($P < 0.05$) coefficients in the equation. In this study final equation was considered with maximum adjusted r^2 value. The adjusted r^2 value for responses Y_1, Y_2, Y_3 and Y_5 were found to be 79.97, 80.51, 84.73 and 88.27% respectively, which indicate good fit. When ANOVA was performed at 95% confidence interval to estimate the significance of the model, factors X_1 (HPMC) and X_3 ($NaHCO_3$) were identified as critical influencing parameters for selected responses Y_1, Y_2 and Y_3. Another response, Y5, i.e. the total floating time was found to be affected significantly by the amount of HPMC (X_1) and CP (X_2) whilst no significant effect of formulation variables was observed on Y4, i.e. floating lag time.

$$Y_1 = 111.946 - 1.518X_1 - 0.195X_3 + 0.009 \cdot X_1^2 \quad (1)$$

$$r^2 = 86.41\%,\ r^2(\text{adjusted}) = 79.97\%$$

$$Y_2 = 169.080 - 1.456X_1 + 0.587X_2 - 1.351X_3 + 0.004X_1^2 - 0002X_1X_2 + 0.012X_1X_3 - 0.002X_2^2 \quad (2)$$

$$r^2 = 87.43\%,\ r^2(\text{adjusted}) = 80.51\%$$

$$Y_3 = 173.766 - 1.130X_1 + 0.908X_2 - 1.775X_3 + 0.005X_1^2 - 0008X_1X_2 + 0.014X_1X_3 - 0.003X_2^2 + 0.004X_2X_3 \quad (3)$$

$$r^2 = 86.39\%,\ r^2(\text{adjusted}) = 84.73\%$$

$$Y_4 = 932.302 - 5.271X_1 - 19.122X_3 + 0.031X_1^2 + 0.127X_3^2 \quad (4)$$

$$r^2 = 37.53\%,\ r^2(\text{adjusted}) = 20.50\%$$

$$Y_5 = -121.083 + 0.949X_1 + 0.294X_2 + 2.042X_3 - 0.005X_1^2 - 0.002X_2^2 - 0.014X_3 \quad (5)$$

$$r^2 = 92.46\%,\ r^2(\text{adjusted}) = 88.27\%$$

3.4. *Formulation optimization and evaluation*

For formulation optimization, target values for the responses ($Y_1 - Y_5$) were set on the basis of *in vitro* drug release and buoyancy study of marketed product, "Zanocin OD" (Table 4). Composition of the optimized formulation given by the software using this technique termed as "multiple response optimization" is shown in Table 5. This combination maximized the desirability over the indicated region to 0.93 (Fig. 1).

Optimized formulation had good physicochemical properties and was found to fulfill the requirement of an optimum

Table 4 – Multiple response optimization.

Response	(Range observed in F1 – F15)		Target value
% Cumulative release at at the end of,	Low	High	
(i) 2 h	29.91 ± 3.97	61.64 ± 3.51	37.08
(ii) 8 h	53.98 ± 4.59	96.70 ± 4.14	77.99
(iii) 12 h	68.99 ± 5.47	100.00 ± 00	92.03
Floating lag time (s)	1.00 ± 00	156.00 ± 10	Minimize
Total floating duration (h)	0.50 ± 0.10	16.00 ± 00	Maximize

Table 5 – Composition of optimized formulation.

Composition	Amount per tablet (mg)	% per tablet weight
Ofloxacin	400.00	49.38
HPMC K100M	66.02	8.15
Crosspovidone	107.88	13.32
$NaHCO_3$	67.17	8.29
Citric acid	30.00	3.70
PVP K30	40.00	4.94
Magnesium Stearate	15.00	1.85
Lactose	83.93	10.36
Total	810.00	100.00

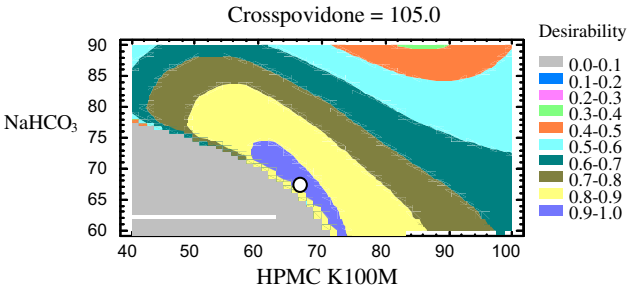

Fig. 1 – Counter plot showing effect of HPMC K100M and $NaHCO_3$ on desirability factor.

controlled release formulation because of better regulation of drug release at the end of 2, 8 and 12 h. The predicted release profile, given by the software was found to be quite close to the profile obtained experimentally which indicates the validity of the developed model (Table 6). Floating lag time of optimized formulation was 2.56 s and it remained floated for more than 16 h *in vitro*. Thus, besides controlling drug release for an extended duration, the formulation seemed to have an excellent floating potential which is prerequisite for prolong residence of the dosage form in the stomach.

The respective release profiles of the marketed and optimized formulation superimposed over each other which indicates analogy of their release performances (Fig. 2). Comparison of release profile of optimized and marketed formulations gave similarity factor ($f2$) of 79.22 and difference

Table 6 – Response variables and release kinetics of marketed product and optimized formulation (observed and predicted).

Formulation	Response variables					Higuchi model		n
	Y_1 (%)	Y_2 (%)	Y_3 (%)	Y_4 (s)	Y_5 (h)	KH	r^2	
Marketed	37.08	77.99	92.02	2.00	>16	27.10	0.98	0.55
Optimized (predicted)	37.08	77.06	93.82	2.72	17.36	23.45	0.99	0.52
Optimized (observed)	39.77	76.09	89.89	2.56	>16	26.01	0.99	0.52

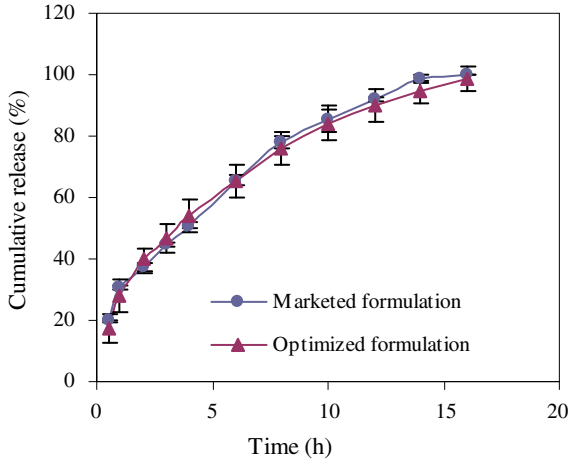

Fig. 2 – Comparative release profile of marketed and optimized formulation.

factor ($f1$) of 3.84. Table 6 shows the comparison of the response variables and other release parameters of these formulations. Percentage cumulative drug release at 2 (Y_1), 8 (Y_2) and 12 h (Y_3) from optimized formulation were found to be quite close to that of marketed product. Buoyancy parameters like floating lag time (Y_4) and total floating duration (Y_5) of these formulations were also lying in close proximity to each other (Table 6). Drug release from both optimized and marketed formulation followed Higuchi's release kinetics and the values of n were found to be 0.52 and 0.55 respectively suggesting the release mechanism to be anomalous transport.

3.5. *Pharmacokinetic evaluation*

The mean serum concentration versus time profiles of the optimized and marketed formulations were similar and

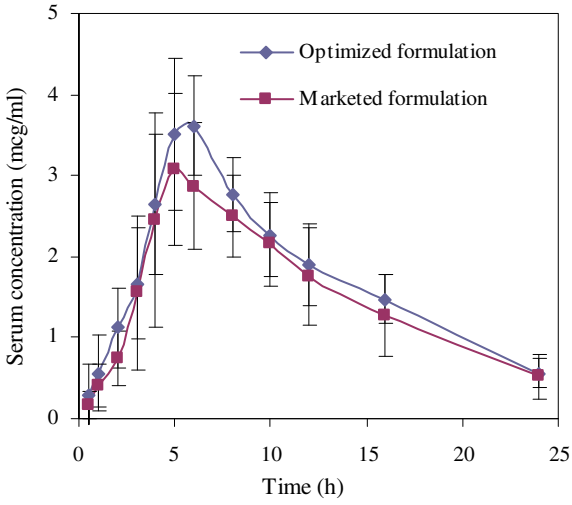

Fig. 3 – Overlain mean serum ofloxacin concentration–time profiles after oral single dose administration of optimized and marketed formulation in 12 healthy human volunteers.

Table 7 – Pharmacokinetic profiles of optimized and marketed formulation.

Parameters	Marketed formulation	Optimized formulation	P value
C_{max} (μg/ml)	3.47 ± 0.70	3.94 ± 0.39	0.04
AUC_{0-24} (μg h/ml)	36.85 ± 4.77	41.80 ± 4.83	0.03
$AUC_{0-\infty}$ (μg h/ml)	43.33 ± 8.82	47.94 ± 6.41	0.06
t_{max}(h)	5.67 ± 1.97	5.75 ± 1.48	–
$t_{½}$(h)	7.36 ± 2.57	7.20 ± 1.52	–
K_{el}	0.10 ± 0.32	0.10 ± 0.02	–

nearly superimposable (Fig. 3). Pharmacokinetic parameters of these formulations are presented in Table 7.

90% confidence interval of the ratio of mean AUC_{0-24}, $AUC_{0-\infty}$ and C_{max} of the optimized to marketed formulation were found to be in the range of 94.27%–103.85%; 99.97%–110.11% and 98.59–111.86% respectively. These values are within the bioequivalence accepted range of 80%–125% [30,31]. Thus, the two formulations can be regarded bioequivalent and hence interchangeable.

4. Conclusions

Controlled release floating gastroretentive tablet dosage form of ofloxacin was successfully developed using Box–Behnken statistical design. Serum drug level monitoring in human subjects for 24 h showed extended drug release for sufficiently longer duration making its once daily administration sufficient. Serum ofloxacin concentration was maintained well above the reported minimum inhibitory concentrations for most of the pathogens for longer duration. The optimized formulation, when compared to the conventional immediate release preparation, seems to be promising for improving bioavailability of ofloxacin for enhancing its therapeutic efficacy along with improving patient convenience due to less frequent dosing requirement.

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REFERENCES

[1] Moes AJ. Gastroretentive dosage forms. Crit Rev Ther Drug Carrier Syst 1993;10:143–195.
[2] Baumgartner S, Kristl J, Vrecer F. Optimization of floating matrix tablets and evaluation of their gastric residence time. Int J Pharm 2000;195:125–135.
[3] Hoffman A. Pharmacodynamic aspects of sustained release preparations. Adv Drug Deliv Rev 1998;33:185–199.
[4] Klausner EA, Lavy E, Friedman M, et al. Expandable gastroretentive dosage forms. J Control Release 2003;90:143–162.
[5] Shaha SH, Patel JK, Pundarikakshudu K. An overview of a gastroretentive floating drug delivery system. Asian J Pharm Sci 2009;4:65–80.
[6] Rouge N, Allemann E, Gex-Fabry M, et al. Comparative pharmacokinetic study of a floating multiple-unit capsule, a high-density multiple-unit capsule and an immediate-release tablet containing 25 mg atenolol. Pharm Acta Helv 1998;73:81–87.
[7] Singh BN, Kim KH. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J Control Release 2000;63:235–239.
[8] Kumar Jeetendra, Shaik MM, Kathi MM, et al. Prescribing indicators and pattern of use of antibiotics among medical outpatients in a teaching hospital of central Nepal. J College Med Sci Nepal 2010;6:7–13.
[9] Chavanpatil Mahesh, Jain Paras, Chaudhari Sachin, et al. Development of sustained release gastroretentive drug delivery system for ofloxacin: *in vitro* and *in vivo* evaluation. Int J Pharm 2005;304:178–184.
[10] Cui Yue, Zhang Yu, Tang Xing. *In vitro* and *in vivo* evaluation of ofloxacin sustained release pellets. Int J Pharm 2008;360:47–52.
[11] Timmermans J, Moes AJ. How well floating dosage do forms float? Int J Pharm 1990;62:207–216.
[12] Hwang SJ, Park H, Park K. Gastric retentive drug-delivery systems. Crit Rev Ther Drug Carrier Syst 1998;15:243–284.
[13] Ritger PL, Peppas NA. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. J Control Release 1987;5:37–42.
[14] Talukder MM, Michael A, Rombaut P, et al. Comparative study on xanthum gum and HPMC as matrices for controlled release drug delivery. Int J Pharm 1996;129:231–241.
[15] Okeri Henry A, Arhewoh Ikhuoria M. Analytical profile of the fluoroquinolone antibacterials. I. Ofloxacin. African J Biotech 2008;7:670–680.
[16] Gu TH, Chen SX, Zhu JB. Phamacokinetics and pharmacodynamics of diltiazem floating tablets. Yao Xue Xue Bao 1992;13:527–531.
[17] Ichikawa M, Watanabe S, Miyake S. A new multiple-unit oral floating dosage system. I: preparation and in vitro evaluation of floating and sustained-release characteristics. J Pharm Sci 1991;80:1062–1066.
[18] Wei Z, Yu Z, Bi D. Design and evaluation of a two-layer floating tablet for gastric retention using cisapride as a model drug. Drug Dev Ind Pharm 2001;27:469–474.
[19] Gambhire MN, Ambade KW, Kurmi SD, et al. Development and *in vitro* evaluation of an oral floating matrix tablet formulation of diltiazem hydrochloride. AAPS PharmSciTech 2007 Sep 7;8(3):E73.
[20] Nagarwal Ramesh C, Ridhurkar Devendra N, Pandit JK. *In vitro* release kinetics and bioavailability of gastroretentive cinarizine HCl tablets. AAPS PharmSciTech 2010;1:294–302.
[21] Jagdale Swati C, Ghorpade Swapnil A, Kuchekar Bhanudas S, et al. Effect of polymer and gas forming agent on floating drug delivery of tramadol hydrochloride using response surface methodology: *in vitro* and *in vivo* evaluation. Int J Pharm Appl 2011;2:181–194.
[22] Tadros Mina Ibrahim. Controlled release effervescent floating matrix tablets of ciprofloxacin hydrochloride: development, optimization and *in vitro* evaluation in healthy human volunteers. Eur J Pharm Biopharm 2012;74:332–339.

[23] Sung KC, Nixon Phillip R, Skoug John W, et al. Effect of formulation variables on drug and polymer release from HPMC-based matrix tablets. Int J Pharm 1996;142:53–60.
[24] Siepmann J, Peppas NA. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose. Adv Drug Deliv Rev 2001;48:139–157.
[25] Reynolds TD, Gehrke SH, Hussain AS. Polymer erosion and drug release characterization of hydroxypropyl methylcellulose matrices. J Pharm Sci 1998;87:1115–1119.
[26] Mitchell K, Ford JL, Armstrong DJ. The influence of drugs on the properties of gels and swelling characteristics of matrices containing methylcellulose or hydroxypropyl methylcellulose. Int J Pharm 1993;100:165–173.
[27] Mitchell K, Ford JL, Armstrong DJ, et al. The influence of the particle size of hydroxypropyl methylcellulose K15M on its hydration and performance in matrix tablets. Int J Pharm 1993;100:175–179.
[28] Siepman J, Kranz H, Bodmeir R. HPMC-Matrices for controlled drug delivery: a new model combining diffusion, swelling, and dissolution mechanisms and predicting the release kinetics. Pharm Res 1999;16:1748–1756.
[29] Prabhakaran L, Vishalini M. Hydrophilic polymers matrix systems of nifedipine sustained release matrix tablets: formulation optimization by response surface method (Box–Behnken technique). Pegalia Res Lib 2010;1:147–165.
[30] FDA. Guidance on statistical procedures for bioequivalence studies using a standard two-treatment crossover design. Rockville, Maryland: Office of Generic Drugs, Center for Drug Evaluation and Research, Food and Drug Administration; 1992.
[31] FDA. Guidance for industry on statistical approaches to establishing bioequivalence. Rockville, Maryland: Center for Drug Evaluation and Research, Food and Drug Administration; 2001.